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New records of *Pezizales* from Pakistan

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Abstract — *Geopora cooperi* f. *cooperi* and *Peziza succosa* have been described and illustrated morphologically and by amplifying ITS1-5.8S-ITS2 region of rDNA. Both taxa are new records from Pakistan.

Key words — ascocarp, ascospores, operculate ascus

Introduction

Pezizales are characterized by unitunicate asci that generally open by rupturing to form a terminal or eccentric lid or operculum (Hansen & Pfister 2006). Of the 1638 pezizalean species known, *Geopora* Harkn. and *Peziza* Dill. ex Fr. are represented by 13 and 104 species respectively (Kirk et al. 2008). From Pakistan three *Geopora* species — *G. arenicola* (Lév.) Kers, *G. arenosa* (Fuckel) S. Ahmad, *G. foliacea* (Schaeff.) S. Ahmad) — and eight *Peziza* species — *P. badiofusca* (Boud.) Dennis, *P. cerea* Bull., *P. gerardii* Cooke, *P. micropus* Pers., *P. pakistanica* (S. Ahmad) S. Ahmad, *P. repanda* Pers., *P. vesiculosa* Bull., *P. violacea* Pers.) — have been reported (Ahmad 1978; Ahmad et al. 1997).

This paper describes two taxa of *Pezizales* newly recorded from Pakistan. The specimens were first identified morphologically, after which the ITS1+5.8S+ ITS2 rDNA regions were amplified for comparison with GenBank sequences of related species. Eighty-seven taxa of *Pezizales* were previously reported from Pakistan; the addition of our recently collected *Geopora cooperi* f. *cooperi* and *Peziza succosa*) raises that total to 89.

Materials & methods

Collected ascocarps were photographed and necessary ecological data were recorded in field. The specimens were dried with the help of fan heater and preserved with tentative field No., for further studies. Apothecia were rehydrated and free hand sections were made in the laboratory. Microscopic (morpho-anatomical) characters were noted at 10× and 40× and drawings were made with the help of camera lucida.

DNA was extracted from dried ascomata, using Extract N. Amp.™ Plant kit (SIGMA). nrDNA of the ITS1-5.8S-ITS2 was amplified using fungal specific and universal primers pairs (ITS1F/ITS4 and ITS1/ITS4, respectively) under the following parameters: denaturation at 94°C for 4 min, then with 35 cycles of 45 s at 94°C, 45 s at 54°C and 1 min 30 s at 72°C, and a final amplification at 72°C for 2 min. Bidirectional sequencing of the purified PCR products was done by Macrogen Inc. (South Korea). ITS sequences were compared using BLAST in GenBank to confirm our identifications.

The specimens have been deposited in the *Pezizales* collection of LAH Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan. Sequences have been accessioned in GenBank.

Taxonomy

Geopora cooperi Harkn., Bull. Calif. Acad. Sci. 1: 168 (1885), **f. *cooperi*** PLATE 1

ASCOCARP: 5–5.5 cm broader, globose to oblong, irregularly convoluted, outer surface dark brown and covered with light brown hairs, inner surface pinkish white to white, highly convoluted, convolutions close but separate. HYMENIUM: 200–250 µm thick. ASCI: Long cylindrical, operculate, centric operculum, unitunicate, 8-spored, uniseriate, non-amyloid, tapering at base, (213–) 221–260 × (16–)19–21 µm. ASCOSPORES: Broadly oval-elliptical, hyaline, smooth, thin walled, with a large central guttule, cytoplasm dense around the guttule, (19–)20–23(–25) × 13–14 µm. Paraphyses: Long, slender, septate, swollen at the tips, nearly of same level as ascus, apical cell 14.5 µm broad. EXCIPULUM: Ectal excipulum: textura globulosa, 120 µm, 3–4 cells thick, cells oblong to circular in section, smooth, walls thick and dark brown, 24–40 × 18–27 µm, cells on the outside darker than the inner cells. Outer cells of the excipulum forming abundant, hyaline to light brown hairs, that are flexuous, branched, 11–13 µm wide, thick-walled, (walls up to 3 µm). Medullary excipulum of textura intricata, thin walled hyphae, hyaline to light brown.

MATERIAL EXAMINED: **PAKISTAN**: **GILGIT-BALTISTAN**, Fairy Meadows, 3036 m (9960ft) a.s.l., under *Pinus wallichiana* A.B. Jacks., solitary, on ground, 24 July 2010, A.N. Khalid TA-50. (LAH 240710; GenBank JN558642).

COMMENTS: *Geopora cooperi* differs from other epigeous *Geopora* species by producing truffle-like ascocarps with a small basal opening and a heavily folded layer (Tamm et al. 2010). It differs from most other truffle-like fungi because of the forcible discharge of ascospores: truffle spores are usually not forcibly expelled from the ascus. Mycorrhizal interaction of *Geopora cooperi* with *Pinus ponderosa* P. Lawson & C. Lawson and the orchid *Epipactis atrorubens* (Hoffm.) Besser has been established (Sheffereson et al. 2008). *Geopora cooperi* f. *cooperi* differs from *G. cooperi* f. *gilkeyae* Burds. in the narrower oval to elliptical ascospores (Burdsall 1968). Our DNA sequence analyses matched the 625 nucleotide ITS region (ITS1-5.8S-ITS2) from the Pakistani collection with that

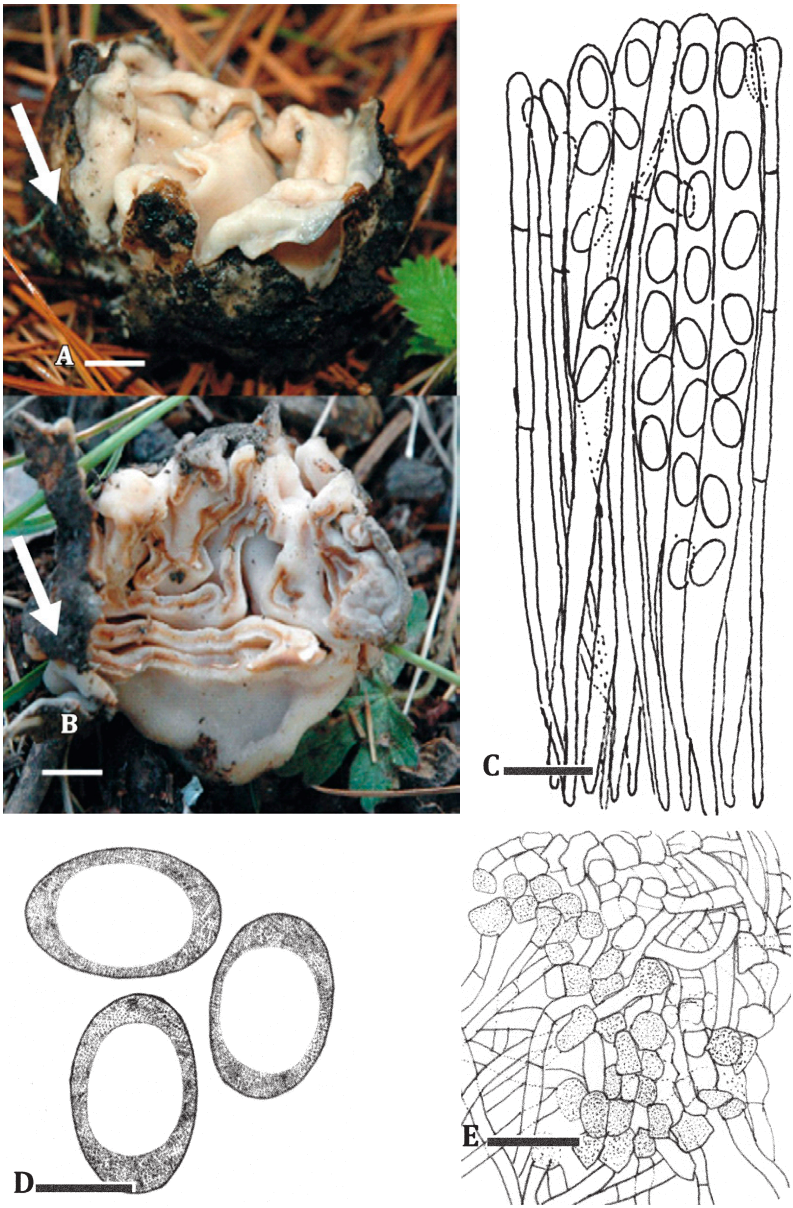

Plate 1. *Geopora cooperi* f. *cooperi*: A–B. Different views of apothecia (arrow indicates outer hairy layer of fruiting body). C. Section of hymenium showing asci and paraphyses. D. Ascospores. E. Ectal excipulum. Scale bars: A = 1.5 cm; B = 1 cm; C = 40 µm; D = 13.5 µm; E = 24 µm

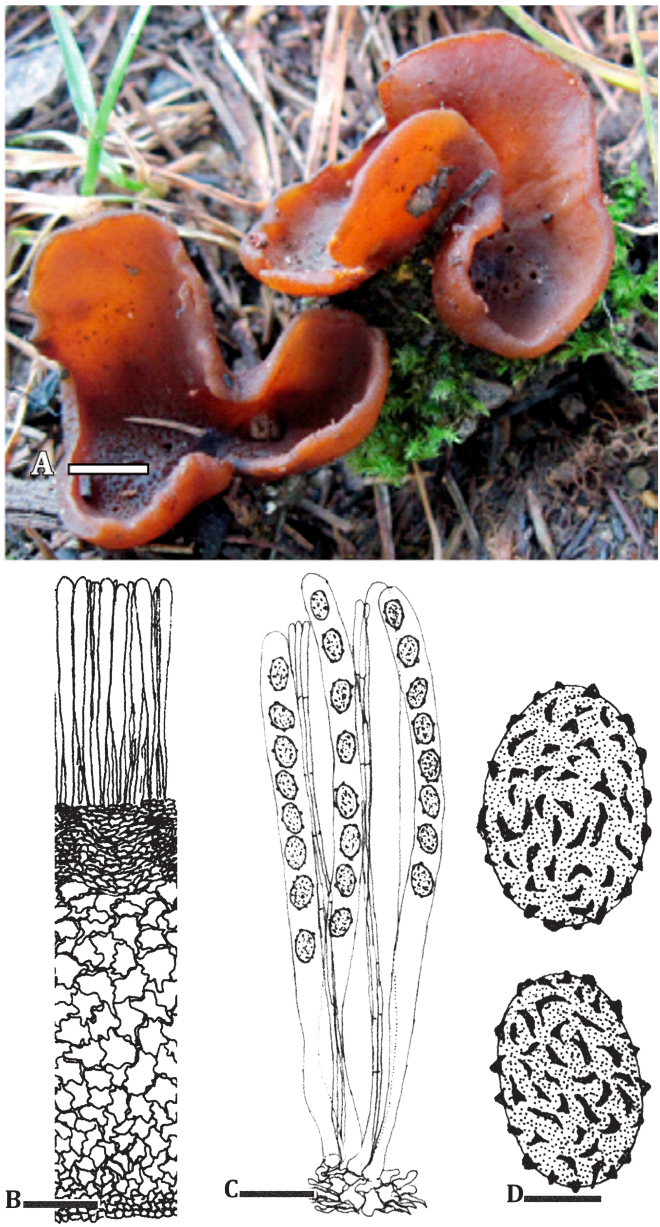

Plate 2. *Peziza succosa:* A. Apothecia. B. Section of apothecium showing hymenium, subhymenium and excipulum. C. Asus and paraphysis. D. Ascospores. Scale bars: A = 1.1 cm; B = 190 µm; C = 49.1 µm; D = 9.5 µm.

from *Geopora cooperi* f. *cooperi* (FR694202.1) at 98% similarity and 90% Query coverage, which we consider sufficient to refer our collection to this taxon.

Peziza succosa Berk., Ann. Mag. Nat. Hist. 6: 358 (1841) PLATE 2

APOTHECIA: Usually gregarious, cupulate, deeply concave, medium-sized, disc concave at first, hemispherical and later slightly irregular, 2.5–3cm in diam., sessile to subsessile, margins entire turning inwards, purplish brown hymenium with slight yellowish tinge, outer surface slightly lighter than hymenium, fresh apothecia exudes yellowish juice when damaged, Subhymenium plectenchymatous. HYMENIUM: up to 304–315 µm. ASCI: Unitunicate, operculate, 8-spored, amyloid at tip, cylindrical slightly narrow near base with flexuous stalk, 260–300 × 17–19µm. ASCOSPORES: Uniseriate, elliptical, ornamented with warts, biguttulate,19–20 × 10–11(–12) µm. PARAPHYSES: Slender, straight, septate, 3.5–4 µm in diam. EXCIPULUM: Ectal excipulum 95 µm thick, textura globulosa to angularis, cells hyaline to slightly brown, smaller thin-walled cells also present; medullary excipulum 285 µm thick epidermoid to polygonal cell, hyaline, thin-walled, 14–25 µm in diam.

MATERIAL EXAMINED: **PAKISTAN**: **KHYBER PAKHTUNKHWA**, Himalayan Moist Temperate Forests, Khanspur, 2575 m (8205 ft) a.s.l., gregarious, on ground, mossy substrate, 21 Aug 2010, T. Ashraf TA-134 (LAH 210810; GenBank JN588568).

COMMENTS: *Peziza succosa* has strongly amyloid asci, with the bluing reaction strongest at the apex, although no pronounced bluing ring is present. With other ectomycorrhizal fungi, *P. succosa* has been reported in deciduous forests and is widespread in Europe, North America (Hansen et al. 2001, Michell 2006), and Asia (DH Pfister, pers. comm.). ITS sequence analyses of our collection showed it to be 99% identical with *P. succosa* (DQ200840.1) from Fredericksburg Have, Zealand, Denmark.

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